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EARLY EFFECTS OF 150-MEV PROTON IRRADIATION IN RHESUS MONKEYS

JOSEPH E. TRAYNOR, Lieutenant Colonel, USAF, MC
ALAN M. SIEGAL, Captain, USAF, MC

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

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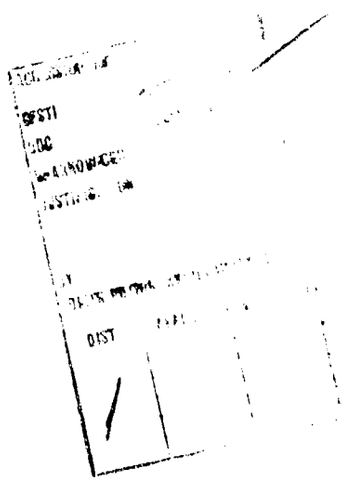

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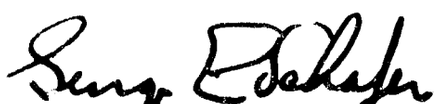
FOREWORD

This report was prepared in the Radiobiology Division under task No. 775704. The research reported herein was funded in part by the National Aeronautics and Space Administration. The work was accomplished between July and November 1967. The paper was submitted for publication on 3 June 1968.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences—National Research Council.

The authors express thanks to the following: the National Aeronautics and Space Administration's Space Radiation Effects Laboratory, for supplying the source of protons; the Texas Nuclear Corporation, for physical dosimetry; the Veterinary Sciences Division, for animal care; the Experimental Branch of the Radiobiology Division, for laboratory support; Major Harold Casey and Captain Philip Coogan, for histopathologic evaluation; Kenneth Hardy, Captain Lawrence Winans, and Master Sergeant Roland Swanson, for technical assistance; and Richard McNee, for assistance with the statistical evaluation.

This report has been reviewed and is approved.



GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

Rhesus primates were exposed to 150-Mev proton irradiation at 11 rads per minute. After exposure, the animals were observed for clinical changes and mortality. Hematologic studies were performed up to 80 days after exposure. The $LD_{50/30}$ was found to be 669 rads. On the basis of acute median lethal dose, mean survival time, clinical observations, and blood cell depression, an RBE of unity was assigned when comparing the 150-Mev proton exposures with 2-Mev x-ray exposures. A decrease in median lethal dose was noted with lowered dose rate when proton exposures at 57 rads per minute and 11 rads per minute were compared.

CORRECTION

("Early Effects of 150-Mev Proton Irradiation in Rhesus Monkeys," by Joseph E. Traynor and Alan M. Siegal, SAM-TR-68-87, Sept. 1968)

The last sentence of abstract, p. iii and in DD Form 1473, should read: "An increase in median lethal dose . . . " (NOT a decrease).

EARLY EFFECTS OF 150-MEV PROTON IRRADIATION IN RHESUS MONKEYS

I. INTRODUCTION

The present study is an evaluation of the early effects of 150-Mev proton irradiation delivered at 11 rads per minute to rhesus monkeys. The results of this study are to be compared with data obtained in previous studies using 2-Mev x-rays delivered at 11 rads per minute (1) and 138-Mev protons at 57 rads per minute (2).

II. MATERIALS AND METHODS

Ninety-six rhesus primates *(Macaca mulatta)* were used in this study; there were 52 males and 44 females. Through a random process of selection, a group of 80 animals was divided into six dose-level groups of 10 to 15 animals each for use in the determination of the $LD_{50/30}$. A second group of 16 animals was divided into four dose-level groups of 4 animals each. The latter were used to study the hematologic effects only and were not included in the calculations of median lethal dose.

The synchrocyclotron of the National Aeronautics and Space Administration's Space Radiation Effects Laboratory (NASA-SREL) was used as the source of protons. An aluminum and lead double degrader configuration was used to degrade the nominal 300-Mev proton beam to obtain the desired spatial distribution and energy of the external beam for the animal exposures.

The animals were placed 17.5 feet from the cyclotron beam exit port. At this position, the spatial distribution of the beam was uniform within ± 6% over a vertical distance of 38 cm. The proton beam was extremely stable; the center of the beam did not vary over 1 cm. in any direction. A variable-volume ionization chamber was used for remotely measuring the relative intensity of the flux. Flux values were measured with a Faraday cup, and dose calculations were made using tabulated values of the mass stopping power for muscle (3). The final energy was 146.8 Mev with an assigned error of ± 3%. The dose values were accurate within ± 6% for all primates. Complete dosimetry details are given elsewhere (4).

The animals were placed individually in galvanized wire-mesh cylinders (40 cm. long and 15 cm. in diameter). These cylinders were rotated in the proton beam at 2 r.p.m. during the exposures.

Just prior to the exposures, a few of the animals developed symptoms of loss of appetite and diarrhea. These animals were removed from the experiment. Stool cultures performed on one of the animals subsequently proved to be positive for *Shigella*. As a prophylactic measure, all animals were given 1 gm. of neomycin by way of a stomach tube, followed the next 3 days by 100 mg. Chloromycetin given intramuscularly.

The animals in the group studied for hematologic effects were bled by femoral venipuncture prior to irradiation and on 3, 8, 11, 16, 22, 30, 45, and 80 days after exposure. Hematologic studies included total leukocyte counts, leukocyte differential analysis, platelet counts, and microhematocrits.

During the 30 days after exposure, all of the animals were observed twice daily for clinical changes and mortality. Necropsies were performed on the dead animals.

TABLE I

Summary of the mortality data

Dose (rads)	Number of animals	Number dead in 30 days	Mortality in 30 days (%)	Mean survival time of nonsurvivors (days)
900	10	10	100	10.7
775	15	12	80	12.9
650	15	5	33.3	17.2
525	15	2	13.3	16.7
400	14	1	7.1	18
275	9	0	0	—

III. RESULTS

An $LD_{50/30}$ of 669 rads with 95% confidence limits of 616 to 727 rads was calculated by probit analysis (5) from the mortality data summarized in table I. The equation for the regression is:

$$Y = 0.2087 + 0.00728X$$

where Y is in probits and X is in rads. The chi-square for the regression heterogeneity is 2.552 with 4 degrees of freedom. This is not significant at the .05 level.

The median lethal dose of this study is very close to the $LD_{50/30}$ of 674 rads after 2-Mev x-irradiation at the same dose rate of 11 rads per minute (1). The slope of the probit regression line from the 150-Mev proton study was compared with the slope of the 2-Mev x-ray probit line. The two lines do not differ significantly at the .05 level; that is, the slopes are not different and the $LD_{50/30}$'s are not different.

A similar comparison was made between the 150-Mev proton irradiation at 11 rads per minute and the 138-Mev proton irradiation at 57 rads per minute. The $LD_{50/30}$ of the 138-Mev proton study was 528 rads. At the .05 level of significance, the slopes of the probit regression lines do not differ; however, the $LD_{50/30}$'s do differ significantly at the .05 level of significance.

In figures 1 and 2, the times of death after the 150-Mev proton irradiation are compared with results of the previous studies using 2-Mev x-irradiation and the higher dose rate, 138-Mev proton irradiation.

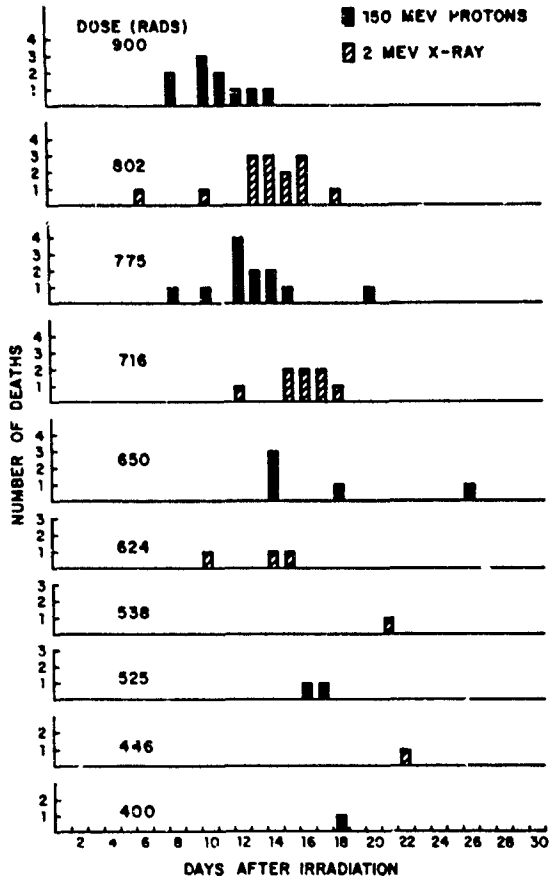


FIGURE 1

Daily mortality after irradiation with 150-Mev protons and 2-Mev x-rays. Both exposures were performed at a dose rate of 11 rads per minute.

The hematologic data are tabulated in tables II to IV. For comparison, the neutrophil and lymphocyte blood levels are shown in figures 3 and 4 with similar dose levels observed in the other 2-Mev x-ray and 138-Mev proton studies. The extent of the depression of these blood elements was similar in all three studies.

Clinically, the animals exhibited moderate malaise with depression of appetite during the first few days after irradiation. By the end of the first postirradiation week, the animals given a higher dose became obviously sick. They sat hunched in their cages and developed symptoms of loss of appetite and diarrhea. Death began to occur in the 900- and 775-rad groups on the 8th day after exposure. About the 12th day after exposure, hemorrhagic diathesis became evident with the appearance of dermal petechiae and, in some cases, patches of ecchymoses. Except for one 650-rad animal, no deaths occurred after the 20th day post-exposure. On the basis of the clinical observations reported in previous irradiation studies, the course of the animals in this study was as anticipated. There was nothing to suspect that these animals were suffering any ill effects from a possible colitis.

The histopathologic results are of special interest. All animals dying within the 30-day

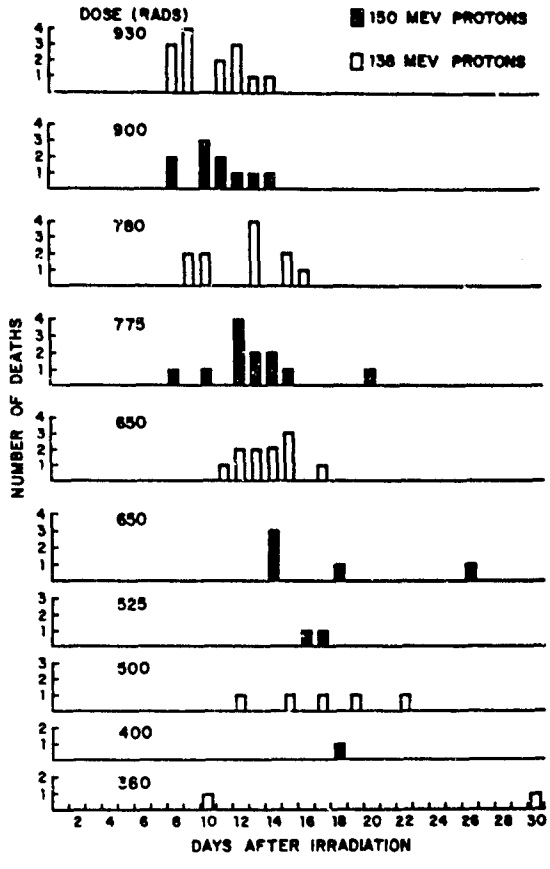


FIGURE 2

Daily mortality after irradiation with 150-Mev protons at 11 rads per minute and 138-Mev protons at 57 rads per minute.

TABLE II

Total white count

Dose group	Days after irradiation								
	Baseline	3	8	11	16	22	30	45	80
Control	9,967	10,125	10,057	9,650	11,495	10,625	10,150	10,300	12,600
275 rads	14,217	6,825	3,283	2,975	3,699	7,250	12,975	14,133	17,300
400 rads	12,275	6,725	2,497	2,675	3,003	10,150	12,625	23,000	13,800
525 rads									
A	11,950	3,300	2,037	2,125	2,693	23,700	19,300	17,350	16,350
S*	14,150	3,600	2,295	2,300	2,693	23,700	19,300	17,350	16,350
N-S*	9,750	3,000	1,778	1,950	——	——	——	——	——

Entries are the average counts, per cubic millimeter, of 4 animals (except the survivor and nonsurvivor subdivisions of the 525-rad group).

A = All animals. S = Survivors. N-S = Nonsurvivors.

*Two animals.

TABLE III

Neutrophils

Dose group	Days after irradiation								
	Baseline	3	8	11	16	22	30	45	80
Control	1,948	5,190	3,228	2,091	3,163	2,227	3,332	2,302	4,293
275 rads	2,784	3,605	1,533	715	1,219	1,212	3,722	5,294	7,592
400 rads	3,465	4,757	1,262	1,113	1,222	3,295	6,794	14,306	5,845
525 rads									
A	2,472	1,575	750	557	431	7,584	6,562	8,136	3,684
S*	2,261	1,773	808	286	431	7,584	6,562	8,1[illegible]	3,684
N-S*	2,183	1,377	691	827	——	——	——	——	——

Entries are the average counts, per cubic millimeter, of 4 animals (except the survivor and nonsurvivor subdivisions of the 525-rad group).

A = All animals. S = Survivors. N-S = Nonsurvivors.

*Two animals.

TABLE IV

Lymphocytes

Dose group	Days after irradiation								
	Baseline	3	8	11	16	22	30	45	80
Control	7,561	6,068	6,649	7,076	7,900	7,769	6,371	7,645	7,948
275 rads	11,020	3,136	1,750	2,254	2,42[illegible]	5,860	8,758	3,112	9,941
400 rads	8,499	[illegible],905	1,229	1,543	1,760	6,711	4,922	8,203	6,996
525 rads									
A	8,998	1,656	[illegible],307	1,573	2,208	15,642	11,194	8,079	11,935
S*	11,223	[illegible],19	1,537	2,003	2,208	15,642	11,194	8,070	11,935
N-S*	6,773	1,594	1,076	1,142	——	——	——	——	——

Entries are the average counts, per cubic millimeter, of 4 animals (except the survivor and nonsurvivor subdivisions of the 525-rad group).

A = All animals. S = Survivors. N-S = Nonsurvivors.

*Two animals.

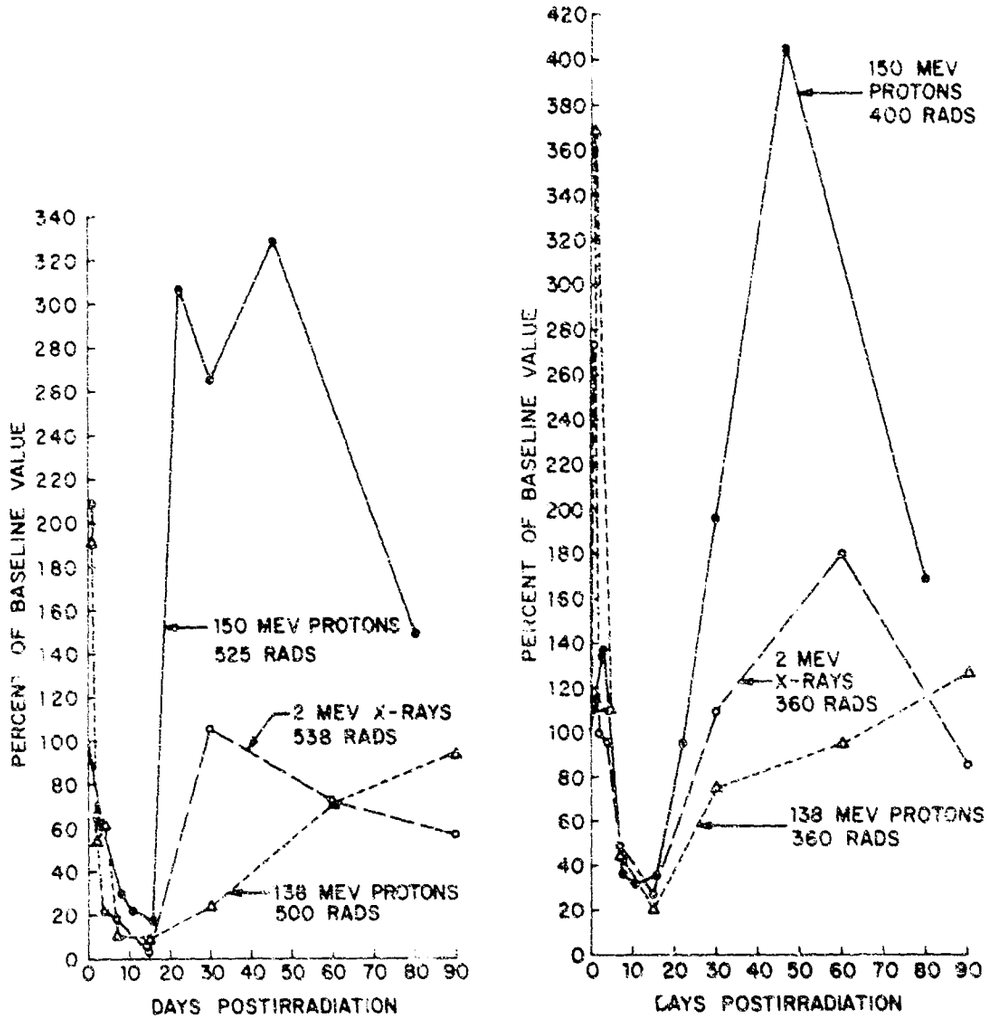


FIGURE 3

Blood neutrophil levels after irradiation, comparing the response of 150-Mev protons and 2-Mev x-rays at 11 rads per minute and 138-Mev protons at 57 rads per minute.

postirradiation period exhibited severe hypoplasia of the bone marrow and lymphoid tissue. Critical evaluation of the colon was made in view of a possible intercurrent *Shigella* infection. Of the animals dying in the acute period after exposure, 50% had some degree of acute colitis consistent with shigellosis. All the cases of colitis were found in the 900-, 775-, and 650-rad groups.

IV. DISCUSSION

Previous reports from this laboratory have suggested an RBE close to unity for various proton exposures; however, the dose rates varied considerably from experiment to experiment. The effect of dose rate on acute mortality is evident when the $LD_{50/30}$ of this study with proton irradiation at 11 rads per minute

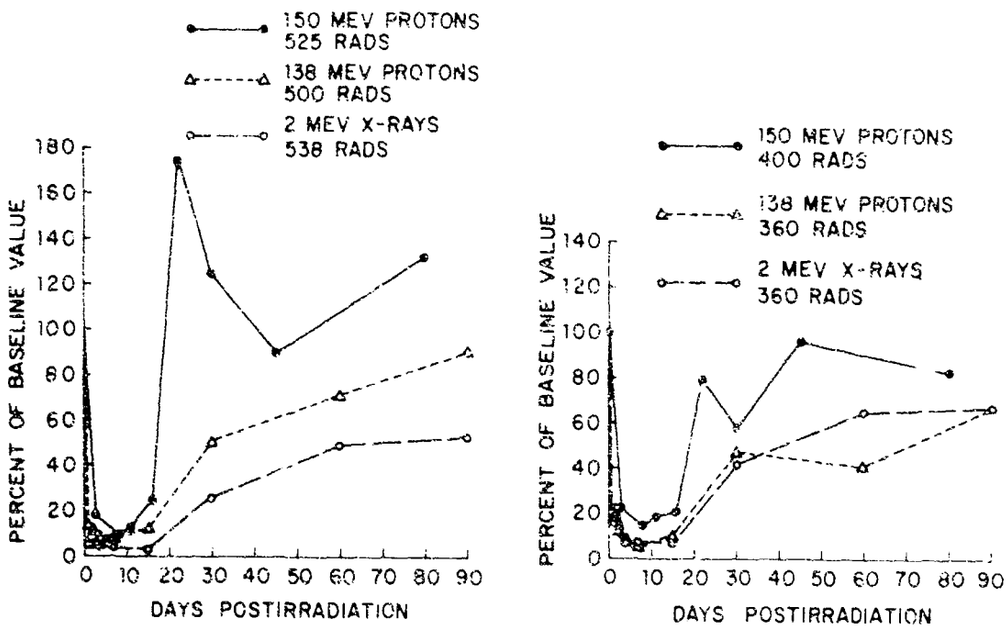


FIGURE 4

Blood lymphocyte levels after irradiation, comparing the response of 150-Mev protons and 2-Mev x-rays at 11 rads per minute and 138-Mev protons at 57 rads per minute.

is compared to the $LD_{50\ 30}$ of a previous proton study at 57 rads per minute. The difference in mortality appears to occur without any marked differences in the extent of blood cell depression; however, the recovery of the peripheral blood cell levels is somewhat delayed at the higher dose rate.

With the present study, it is possible to compare the biologic effects of a proton irradiation with an x-irradiation which involved the same animal species, experimental technics, and dose rates. In the latter comparison, the biologic effects were found to be almost a complete duplication after 150-Mev proton or 2-Mev x-ray exposures. Thus, on the basis of acute median lethal dose, mean survival time, clinical observations, and blood cell depression, an RBE of unity can be assigned.

The intercurrent colitis and the course of antibiotic treatment in the present study must not be overlooked. The clinical course of the animals in the postirradiation period suggested that the spread of the colitis had been checked; however, the histopathologic examinations indicated that this was not true. In the group studied for hematologic effects, the dramatic increase in the white blood cell counts in some of the animals after the third postirradiation week could possibly be a reflection of added hematopoietic stimulation due to infectious organisms.

It could be suggested that the original bacterial infection was subclinical and, along with the antibiotic treatment, the infection was all but eliminated. After exposures, the stress incurred by the irradiation could have allowed a few remaining bacteria, which normally would not have been a problem, to get the upper hand in those animals which had precariously low blood counts. Since the colitis was observed histopathologically in the animals given the highest exposure doses, one could speculate that these animals would have died anyway. It is interesting to note that the

blood cell counts in the control hematology group remained very constant, thus indicating that bacterial infection was not a problem in these animals.

The remarkable thing about this study has been the very close parallel between the findings of this study with proton irradiation and the findings of the study using 2-Mev x-rays at the same dose rate. The authors conclude that there is virtually no difference in the acute biologic manifestations after either 2-Mev x-irradiation or 150-Mev proton irradiation at a dose rate of 11 rads per minute. It is also concluded that a dose-rate effect on acute mortality after proton irradiation does exist.

REFERENCES

1. Dalrymple, G. V., I. R. Lindsay, and J. J. Ghidoni. The effect of 2 Mev whole-body x-irradiation on primates. Radiat. Res. 25:377-400 (1965).

2. Dalrymple, G. V., et al. Some effects of 138 Mev protons on primates. Radiat. Res. 28:471-488 (1966).

3. Janni, J. F. Calculations of energy loss, range, pathlength, straggling, multiple scattering, and the probability of inelastic nuclear collisions for 0.1 to 1000 Mev protons. AFWL-TR-65-150, Sept. 1966.

4. Boles, L. A., et al. Primate irradiations and thermoluminescent dosimetry at the NASA-SREL synchrocyclotron. Contract AF41(609)-67-C-0106, 21 Mar. 1968. (Unpublished)

5. Finney, J. D. Probit analysis. London: Cambridge University Press, 1952.

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Joseph E. Traynor, Lieutenant Colonel, USAF, MC
Alan M. Siegal, Captain, USAF, MC

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13. ABSTRACT

Rhesus monkeys were exposed to 150-Mev proton irradiation at 11 rads per minute. After exposure, the animals were observed for clinical changes and mortality. Hematologic studies were performed up to 80 days after exposure. The $LD_{50/30}$ was found to be 669 rads. On the basis of acute median lethal dose, mean survival time, clinical observations, and blood cell depression, an RBE of unity was assigned when comparing the 150-Mev proton exposures with 2-Mev x-ray exposures. A decrease in median lethal dose was noted with lowered dose rate when proton exposures at 57 rads per minute and 11 rads per minute were compared.

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